Phenotyping Fusarium resistance in oats with National Plant Phenotyping Infrastructure - FUSNAPPI

Juho Hautsalo, Luonnonvarakeskus (Luke)

Lauri Lehtilä, University of Helsinki (UH)

Mirko Pavicic, UH

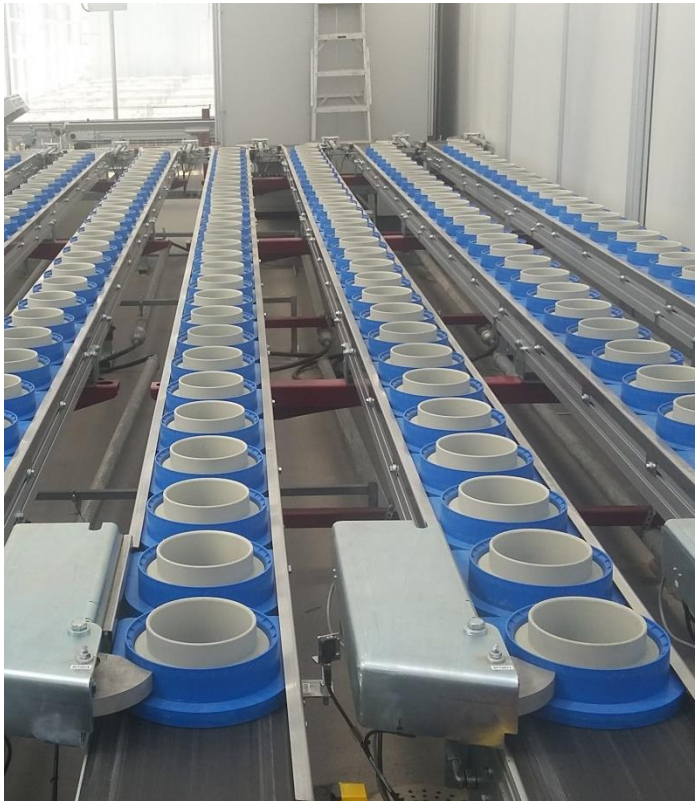
Kristiina Himanen, UH

Marja Jalli, Luke

Luke
NATURAL RESOURCES INSTITUTE FINLAND

Oatline of my presentation

- PhD thesis project
- Fusarium resistance
- Spikelet inoculation method
- FUSNAPPI project
- FUSNAPPI results



Fusarium head blight is a serious problem in oats

- Nordic countries produce and export high quality oats
- In the worst years up to 25% of oat samples can exceed limit for DON mycotoxin
- Also germination and yield are affected by the disease
- Can’t be fully controlled by agricultural practises

Phenotyping Fusarium head blight resistance in oats

- Suitable phenotyping tools and resistance sources were both needed for the breeding of FHB resistant oats.

- Thesis project 2016-2018:

  - Reviewed the current state of Fusarium resistance breeding in oats (Hautsalo et al. 2018, Euphytica)

  - Evaluated testing methods in field and greenhouse

  - Named potential resistance sources and susceptible cultivars

  - Studied traits that can lead to evasion of disease such as earliness and flowering

Fusarim resistance is multicomponential quantitative trait

Genotype

Resistance against what?
I Initial infection (Schroeder & Christensen 1963)
II Spread of infection (Schroeder & Christensen 1963)
III Kernel infection (Mesterhazy, 1995)
IV Tolerance (Mesterhazy, 1995)
V Toxin accumulation (Miller et al. 1985)

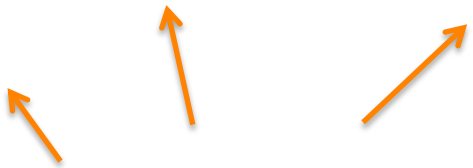
Evasion mechanisms

- Earliness
- Height
- Lodging
- Flowering habit?
- ….

Phenotyped traits:

- Infected grains
- Fungal biomass
- Germination
- Yield loss
- Mycotoxin content
- Symptoms
- ….

Phenotype

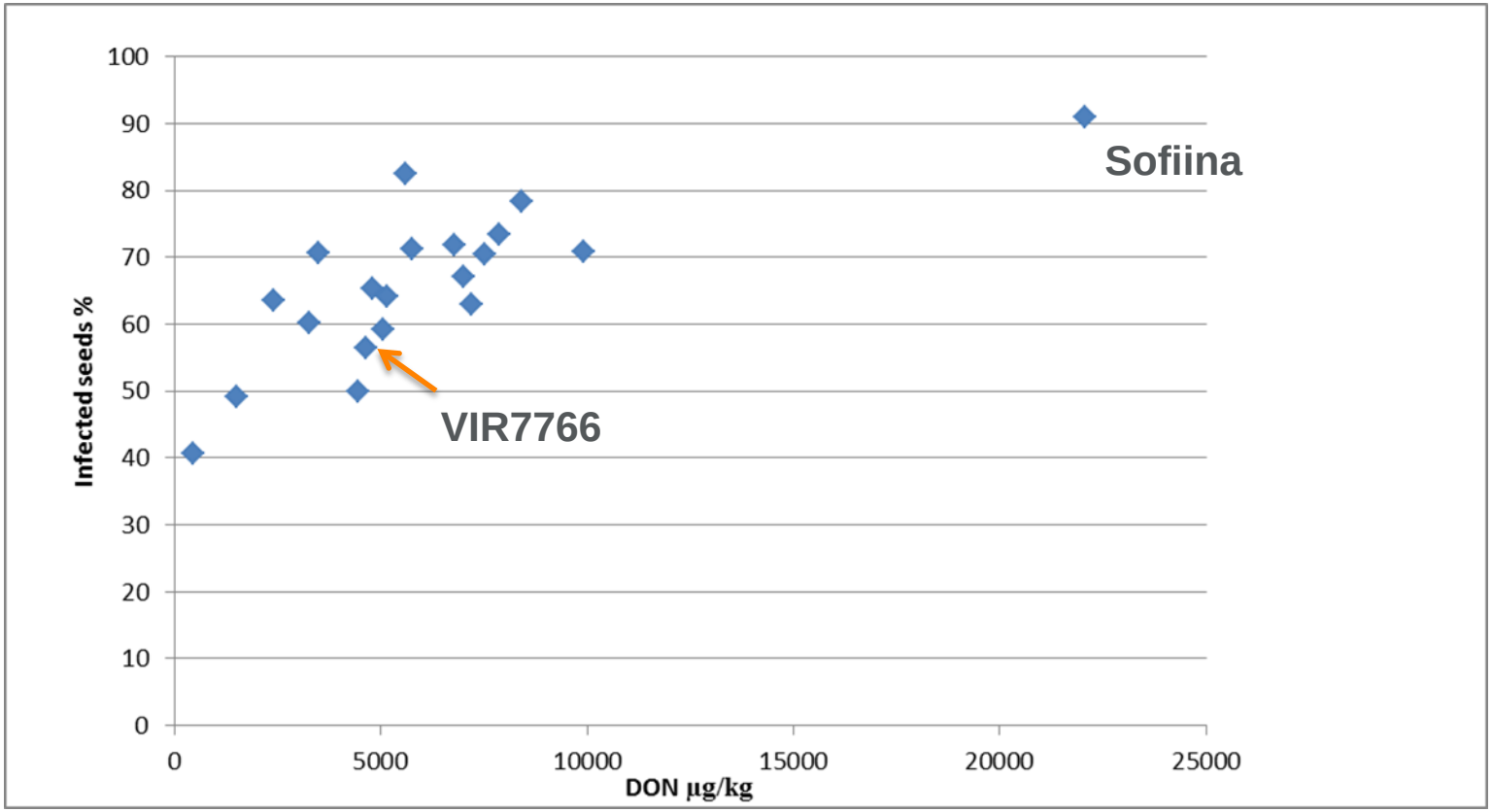


Environment

Greenhouse experiments

Spray inoculations can separate resistant and susceptible oat lines

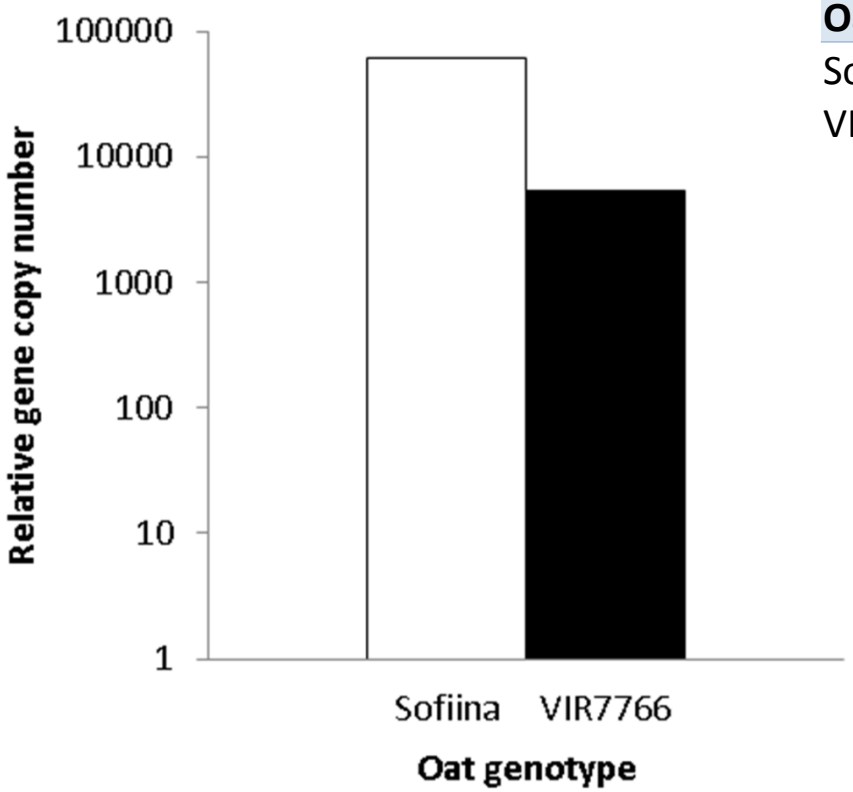
+ Infected kernels have high correlation with DON r=0.68
- Analyses made from yield are quite expensive and experiments often need repetition



Spikelet inoculation in oats

Hautsalo, Latvala, Manninen, Hannukkala, Haapalainen, Jalli

VIR7766 and Sofiina six days after point inoculation done at anthesis



Oat genotypes	Weight reduction (grams)
Sofiina	0.013
VIR7766	0.005

Weight reduction and Fusarium biomass correlate ($R^2=0.56$, $p<0.0001$)
(preliminary data based on 1 experiment)

FUSNAPPI-project

FUSarium detection within National Plant Phenotyping Infrastrucure

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FusNaPPi Project

Lauri Lehtilä

- Small spin-off from my PhD project
  (12/2017-12/2018)
- Aim was to learn to utilize automated greenhouse phenotyping platform for oats and also use imaging technologies in Fusarium resistance screening
- NaPPi-infrastucture enables us to monitor plant physiology with with high-throughput non-destructive methods
- A master's thesis and somekind of peer reviewed publication will be produced from this

National Plant Phenotyping Infrastucture

Further information:
Kristiina Himanen, NaPPi-group leader at University of Helsinki

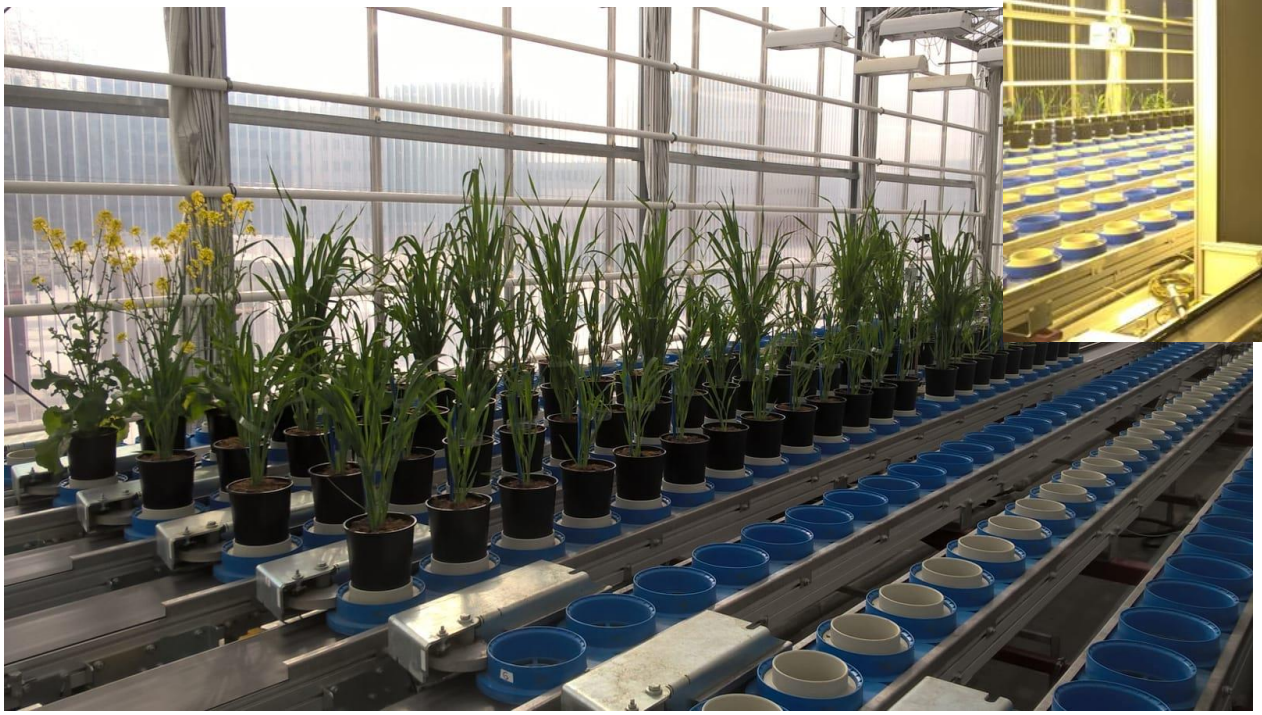
Large plant system:

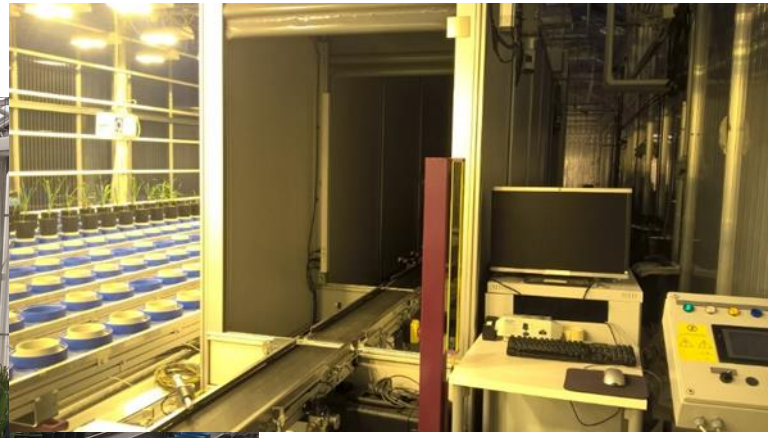
- housed in greenhouse space
- maximum plant screen capacity 270 large plants in 3,5L rose pot OR 5L large pot
- crop plants and trees, maximum 120 cm in height and 100 cm width
- Acclimation chamber for dark or light treatments up to 1000 uE.

Imaging units:

RedGreenBlue (RGB) visible light

PAM chlorophyl fluorescence





Oat grew surprisingly well in the middle of winter!

National Plant Phenotyping Infrastucture

Further information: Kristiina Himanen

Small plant system:

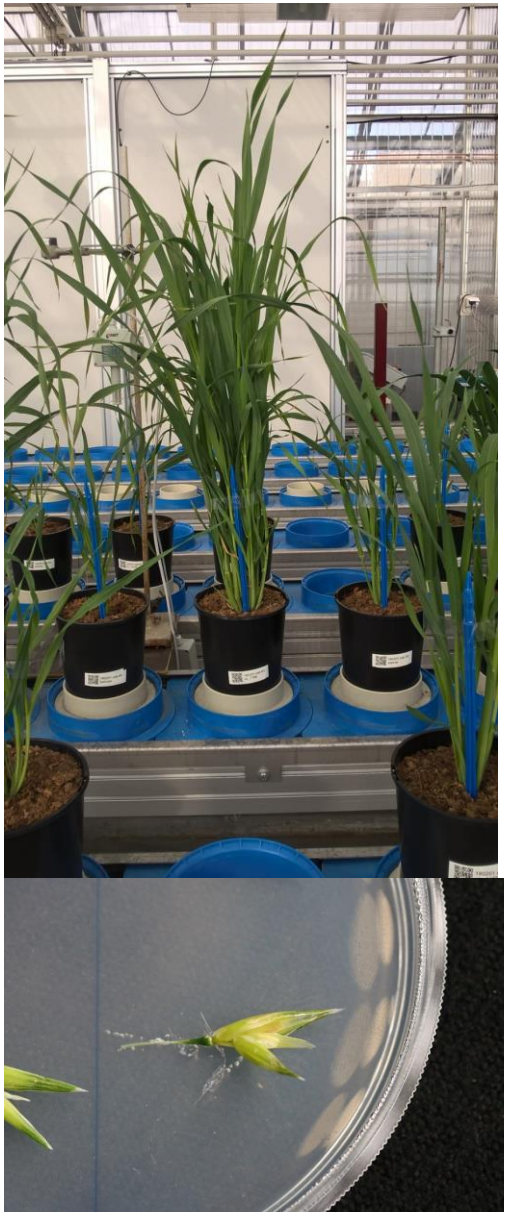
- housed in a temperature and humidity controlled phytoscope
- maximum plant screen capacity for one run is 360 small plants in 6×6 cm pots (20 pots per tray) e.g. Arabidopsis or other small seedlings
- total phytoscope capacity is for 1080 small plants
- variable tray lids allow different plant species to be screened (fewer pots per tray or complete trays)
- maximum height of small plants is 50 cm
- in vitro plates and multi-well plates can also be imaged

Imaging units:

RedGreenBlue (RGB) visible light
Infrared thermal camera (IR)
PAM Chlorophyll Fluorescence

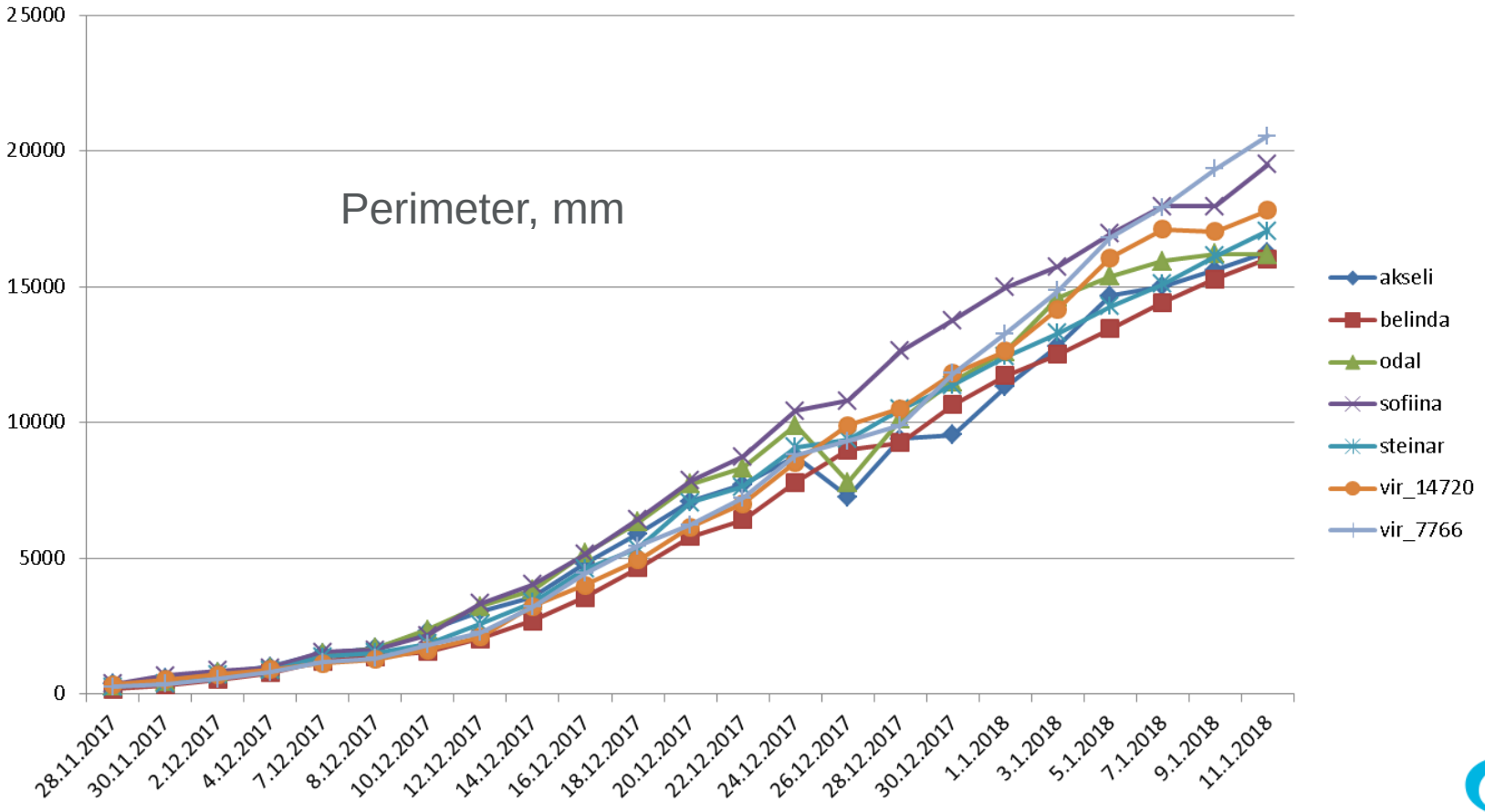
Material and methods

- Seven oat genotypes with different susceptibility to FHB were sown in 3 batches during the sping (each containing 5 replicates)
- Growth and morphology was monitored in the greenhouse phenotyping system (RGB)
- For infection we used an in vitro approach in small NaPPi with spikelet inoculation to avoid contamination
  - spikelets were collected to agar-plates at anthesis and inoculated with *F.graminearum*
  - Two inoculated and mock-inoculated plates per genotype with 8 spikelets
  - RGB and Fluorescence cameras
  - Monitored 1-3 times per day

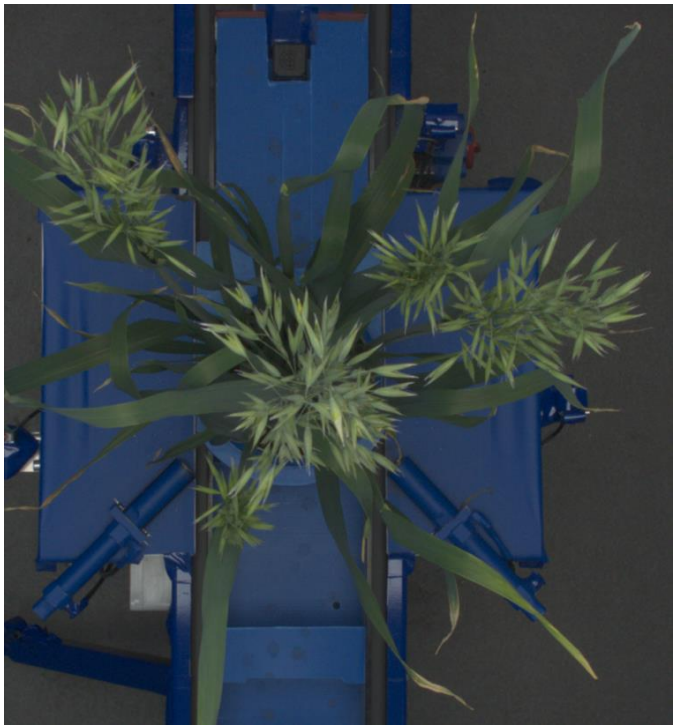


Results from greenhouse

Earliness and growth speed can be compared



Also traits such as oat flowering can be seen from RGB pictures but the system was not fast enough to monitor the daily duration of flowering.

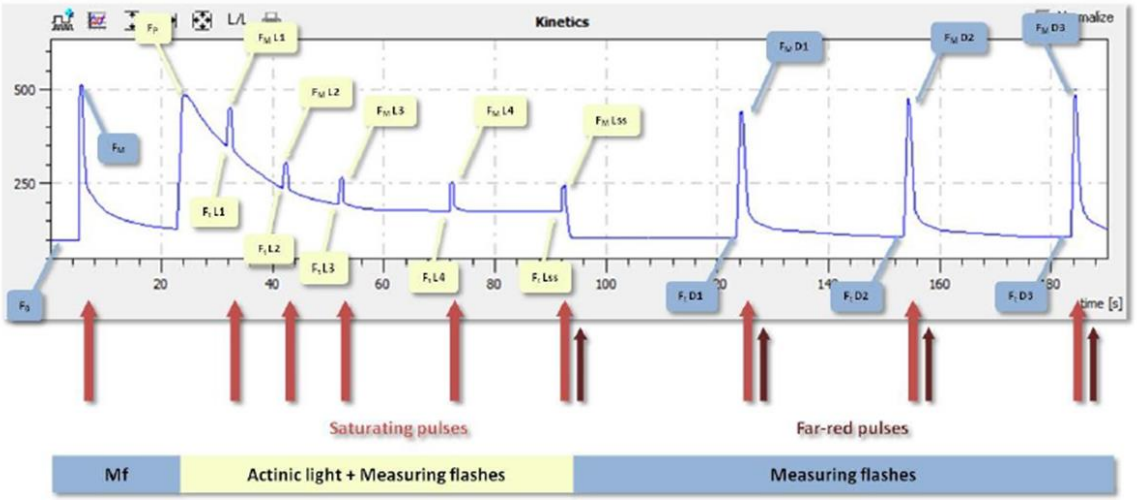


Spreading of infection

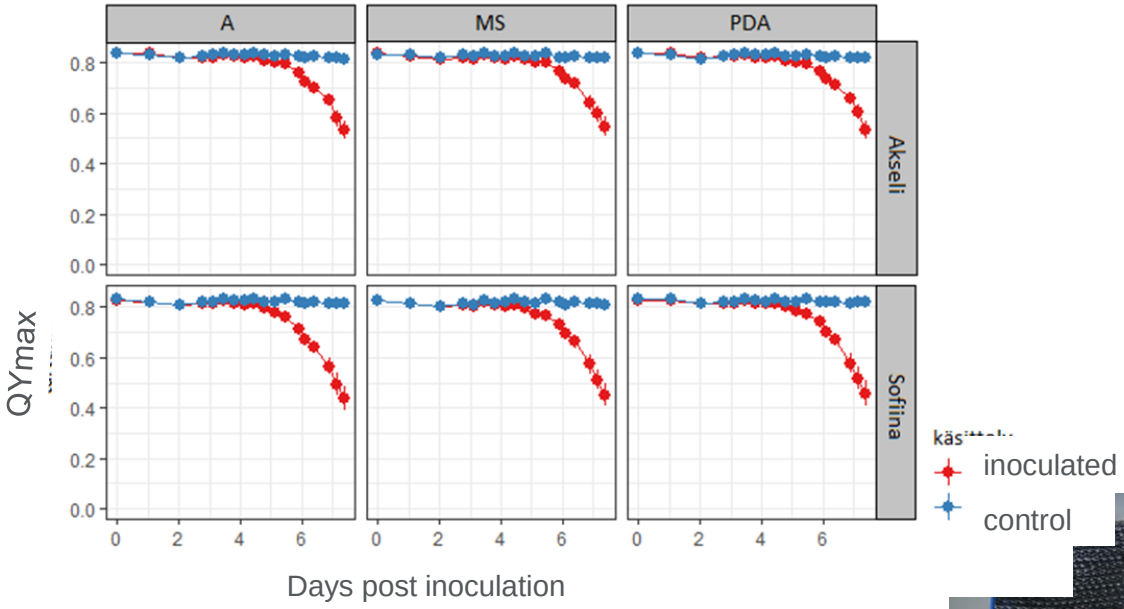
We used chlorophyll fluorescence as a plant health indicator

- **AbsorbedLight= Photosynthesis+ Heat+ Fluorescence**
- One of the most often employed parameters is maximum quantum yield of PSII (Fv/Fm)
- The weakening of photosynthesis measured by fluorescence can be localized into infected plant tissue (Lohaus et al. 2000, Plant Biology, Charte et al. 2005, Plant, Cell and Environment)

Quenching protocol



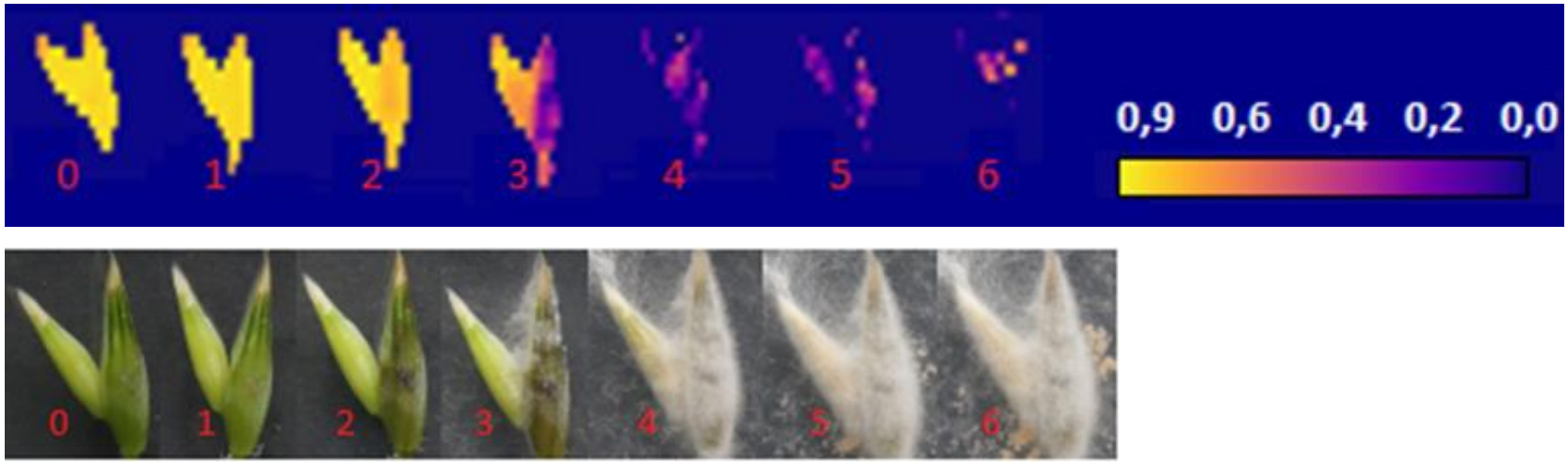
Pre-experiment: Comparison of different agar media with two cultivars



MS medium without added carbohydrates gave the best vision to symptom development and did not promote mycelium growth as much as PDA or water agar

Quantum yield maximum declined slightly faster in oat genotype ’Sofiina’ than ’Akseli’. Media did not differ from each other.

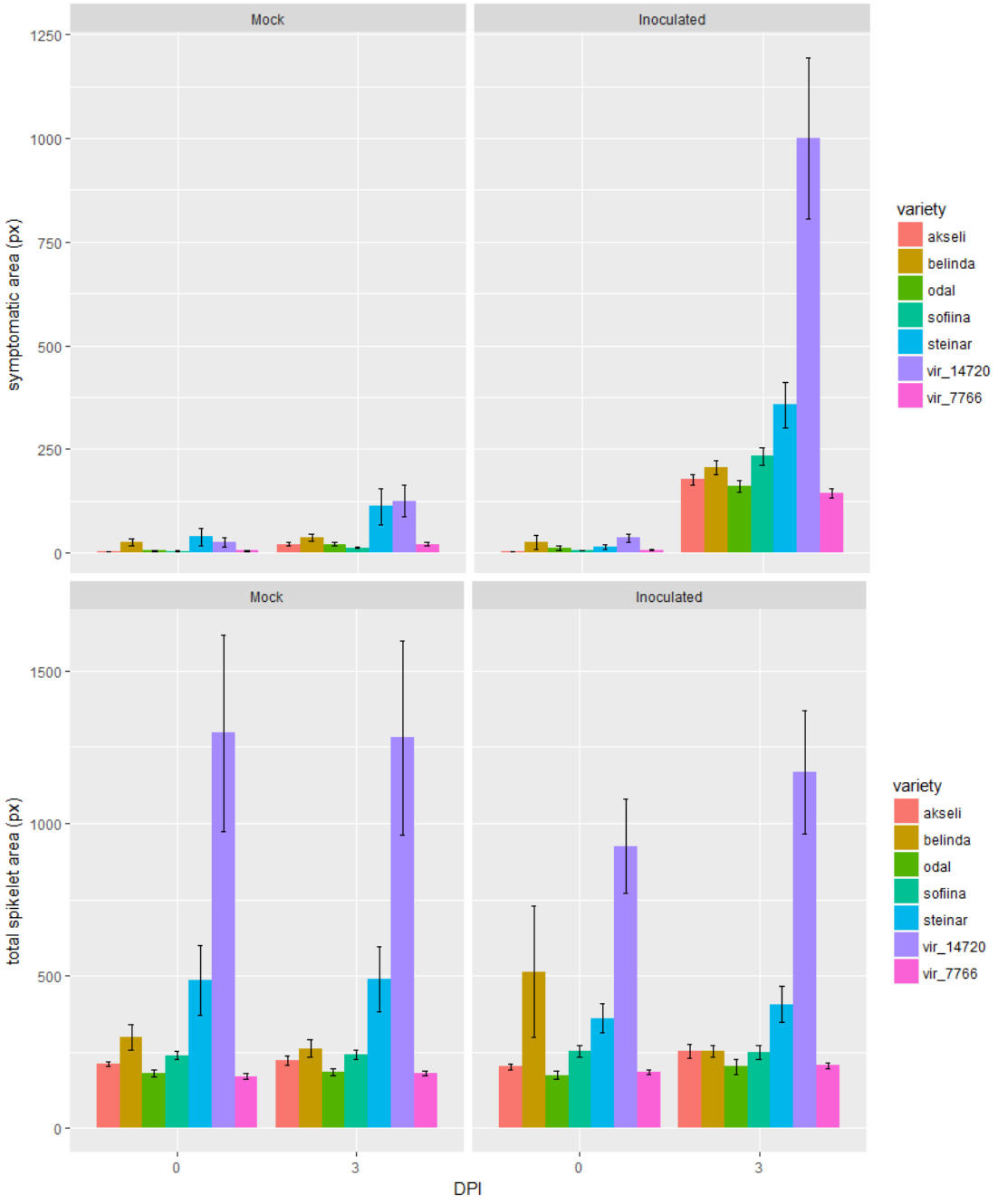
Development of infection and degradation of photosynthesis in oat cultivar ’Odal’



Photosynthesis measured by Quantum yield maximum declined quickly at 4 dpi in all oat genotypes. Mycelium covers the tissue, but differences at day three prove that also photosynthesis machinery is influenced inside the tissue.

Symptomatic area (QYmax decreased) was relative to spikelet size

Hulless oat genotype VIR_14720 had the largest spikelets due to indetermined flowering type

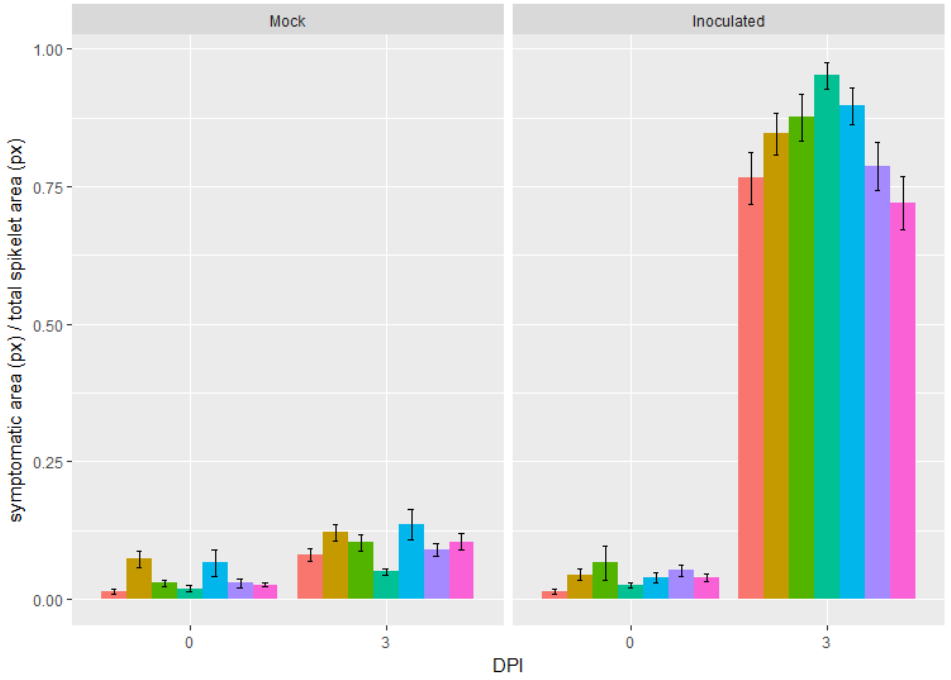


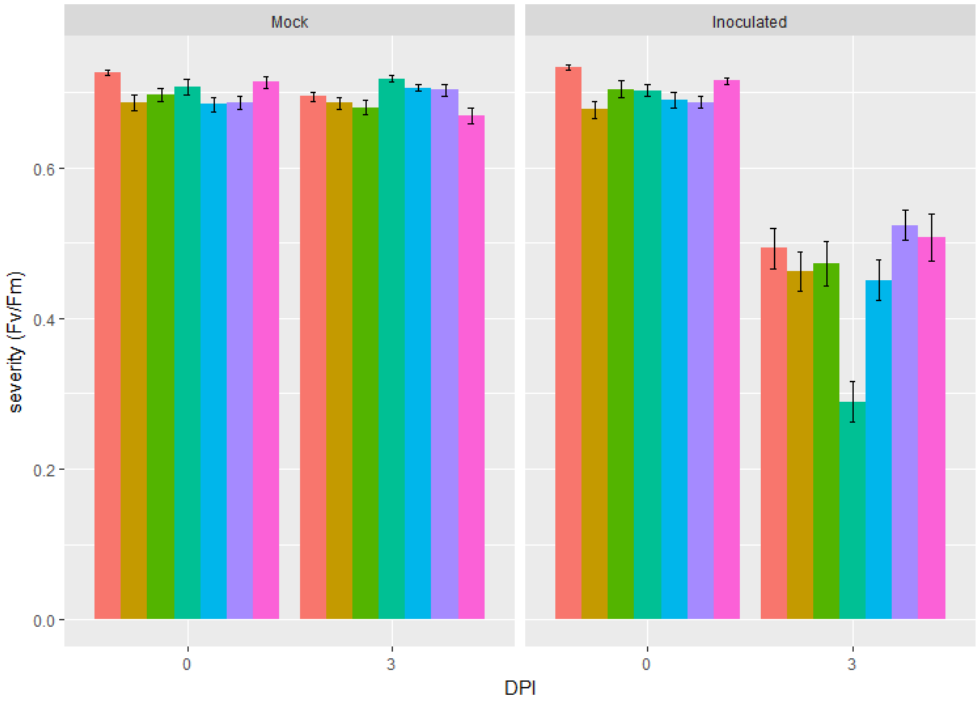
Two ways to detect differences

Symptomatic area is determined as area going under 0.75 in Qymax (Fv/Fm)

Severity measures how bad the decline is within the symptomatic area

These results are nicely in line with other greenhouse data





Unfortunately controlled environment shows only part of the picture

	DON in greenhouse	DON in field
Sofiina	22 ppm	13,5 ppm
VIR7766	4,6 ppm	16 ppm

These estimates are calculated from minimum of four separate field or greenhouse experiments

Bad agronomy of VIR7766 probably leads to susceptibility in Nordic conditions whereas early flowering and maturing Sofiina avoids infection in field.

Conclusions

- In an oat spikelet the infection by *Fusarium graminearum* can lead to development of visual symptoms, deminishing of photosynthesis, significant accumulation of fungal biomass and reduction of fresh weight within six days.

- Genotypic differences indicate that oat lines have measurable resistance mechanisms that play role within the early infection

- Besides the differences in resistance the adaptation to growing conditions can play a significant role in agriculture

- Multiple methods are required to compile Fusarium resistance

Thanks for listening!

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